bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Antisense oligonucleotide and LNA GapmeR transfections

Caroline Jane Ross Igor Ulitsky

Updated date: Jul 24, 2022

An abbreviated version of this protocol was published in Genome Biology in Jan 2021

Uncovering deeply conserved motif combinations in rapidly evolving noncoding sequences

DOI: 10.1186/s13059-020-02247-1

Detailed protocol

Transfection of ASOs in Neuro-2a (N2a) and MCF-7 cell lines:

The following protocol describes seeding density and reagent volumes used to transfect ASOs or Gampers in N2a and MCF-7 cell lines in a 6-well plate. Note that the following protocol is efficient to transfect ASOs and LNA GapmeRs to both final concentrations of 25nM and 50nM. Cells and volumes can be scaled according to plate size and final amount of ASOs used.

1. Both N2a and MCF-7 cell lines were routinely cultured in DMEM containing 10% fetal bovine serum (FBS) and 100 U penicillin/0.1 mg ml^{-1} streptomycin (PS) at 37 °C in a humidified incubator with 5% CO_2
2. The following transfection reagents were used according to the cell-line:
    i. Dharmafect2 for N2a
    ii. Dharmafect4 for MCF-7

1. Transfection steps:
    1. One-day prior to transfection seed 200,000 cells per well in a 6-well plate in a total volume of 2ml DMEM (containing PS and FBS)
    2. On the day of transfection, removed DMEM and wash cells twice with PBS. Replace the medium in each well with 1600ul of Antibiotic free medium (DMEM+FBS)
    3. In a sterile autoclaved eppendorf tube, prepare a 10uM stock of ASOs/Gapmers. ASOs and Gapmers can be diluted in ultra-pure water (DNAse and RNAse free) and stored at -80c
    4. For transfection at a final concentration of 25nM prepare the following two tubes:
        i. Tube 1: 5ul of ASOs (10uM stock) in 195ul filtered OptiMEM. Mix gently by pipetting several times. Incubate at room temperature for 5min.
        ii. Tube 2: 4ul of Dharmafect reagent in 196ul filtered OptiMEM. Mix gently by pipetting several times. Incubate at room temperature for 5min.
    5. After 5min incubation at room temperature, add Tube1 to Tube2. Mix gently by pipetting several times. Incubate at room temperature for 20min.
    6. After 20min incubation add the 400ul transfection mix to cells contained in 1600ul antibiotic free medium.
    7. Incubate at 37 °C in a humidified incubator with 5% CO_2 for 48-72hours.
    8. To prevent contamination medium can be replaced with DMEM containing PS and FBS, 24-hours post transfection.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Ross, C. and Ulitsky, I. (2022). Antisense oligonucleotide and LNA GapmeR transfections. Bio-protocol Preprint. bio-protocol.org/prep1815.
2. Ross, C. J., Rom, A., Spinrad, A., Gelbard-Solodkin, D., Degani, N. and Ulitsky, I.(2021). Uncovering deeply conserved motif combinations in rapidly evolving noncoding sequences. Genome Biology 0(0). DOI: 10.1186/s13059-020-02247-1

Copyright: Content may be subjected to copyright.